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SYNTHESIS OF (±) TYLOPHORININE

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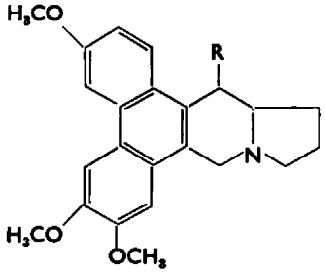
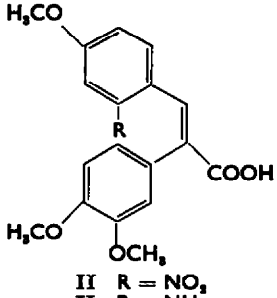
Abstract—Structure I assigned to the alkaloid tylophorinine has been proved by a total synthesis of its racemic form.

IN PREVIOUS communications,[1] experiments leading to the assignment of structure I to tylophorinine, the minor alkaloid of *Tylophora asthmatica* have been detailed. The synthesis of desoxytylophorinine (Ia) obtained by hydrogenolysis of the alkaloid conclusively proves the skeletal structure and the positions of the methoxyl groups. The placement of the alcoholic hydroxyl group is based on the fact that it undergoes hydrogenolysis and should therefore be benzylic. Since tylophorinine does not behave as a carbinolamine, the hydroxyl group is placed in the position shown in structure I. A successful synthesis of the racemic form of the alkaloid according to the scheme reported in this paper proves the correctness of structure I assigned to the alkaloid in all respects.

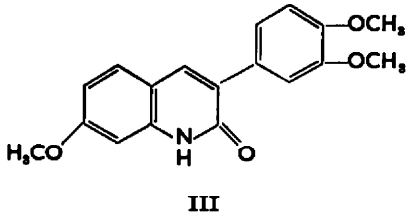


I R = OH
Ia R = H

Earlier,[2] a synthesis of 9-chloromethyl-3,6,7-trimethoxyphenanthrene required in the present scheme was reported from this laboratory. This has now been made by a



II R = NO_2
IIa R = NH_2



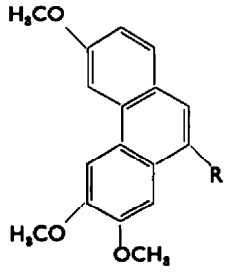
III

* CIBA Research Centre, Goregaon East, Bombay 62.

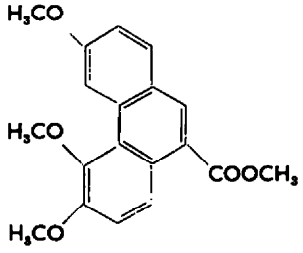
[1] T. R. Govindachari, B. R. Pai, S. Rajappa and N. Viswanathan, *Chem. & Ind.* 950 (1959); T. R. Govindachari, B. R. Pai, I. S. Ragade, S. Rajappa and N. Viswanathan, *Ibid.* 966 (1960); *Tetrahedron* 14, 288 (1961).

[2] T. R. Govindachari, I. S. Ragade and N. Viswanathan, *J. Chem. Soc.* 1357 (1962).

different and superior procedure. Condensation of 4-methoxy-2-nitrobenzaldehyde with homoveratic acid yields 4-methoxy-2-nitro-α-(4,5-dimethoxyphenyl) cinnamic acid (II) which is reduced to the corresponding amino acid (IIa) by ammoniacal ferrous sulphate, the 2-oxoquinoline (III) being a by-product. On being submitted to the Pschorr reaction followed by esterification, the amino acid (IIa) yields a mixture of methyl 3,6,7-trimethoxyphenanthrene-9-carboxylate (IV) and methyl 3,5,6-trimethoxyphenanthrene-9-carboxylate (V) which were separated by taking



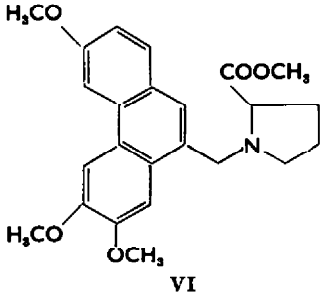
IV R = $COOCH_3$
IVa R = CH_2OH
IVb R = CH_2Cl



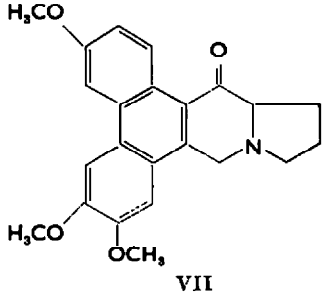
V

advantage of their different solubilities in methanol. Reduction of the ester (IV) with LAH, yields the carbinol (IVa) which is converted to the chloromethyl compound (IVb) by thionyl chloride.

Condensation of 9-chloromethyl-3,6,7-trimethoxyphenanthrene (IVb) with methyl L-prolinate yields methyl 3,6,7-trimethoxy-N(9-phenanthrylmethyl) pyrrolidine-2-carboxylate (VI). This was hydrolyzed to the corresponding acid isolated as the crystalline hydrochloride which was cyclized by polyphosphoric acid at 100° to 9, 11, 12, 13, 13a, 14-hexahydro-3,6,7-trimethoxy-14-oxodibenzo (f,h) pyrrolo (1,2b) isoquinoline (VII).



VI



VII

On reduction with sodium borohydride the ketone (VII) yields a mixture of two main components (TLC). One of these could be obtained in a pure state by column chromatography over alumina (twice) followed by crystallization. The compound m.p. 246° (dec) has an IR spectrum (KBr disc) identical with that of natural (−) tylophorinine. Although L-proline has been used in the synthesis, it is clear that the ketone (VII) should be racemic, since the conditions of its formation are such that optical activity would hardly be retained. Reduction of the ketone should yield a mixture of two racemic diastereoisomers, from which one racemate corresponding to

(−) tylophorinine has been isolated. The complete identity of the solid phase IR spectra of the natural (−) and synthetic (±) compounds should be ascribed to the identity of their crystalline states.

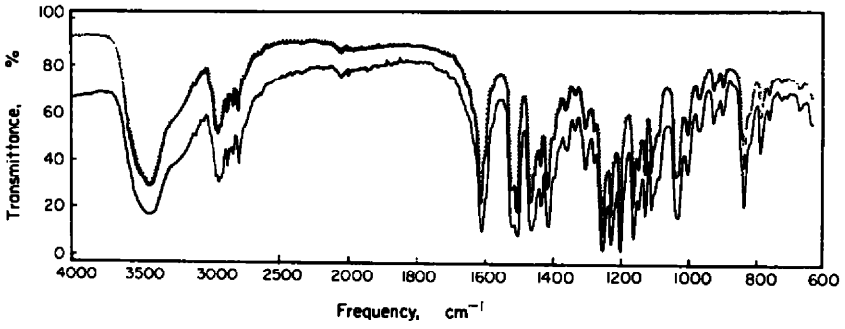


FIG. 1

EXPERIMENTAL

4-Methoxy-2-nitro-α-(4,5-dimethoxyphenyl) cinnamic acid

(a) A mixture of 4-methoxy-2-nitrobenzaldehyde (63·35 g) homoveratric acid (70·56 g), redistilled acetic anhydride (280 ml) and dry triethylamine (42 ml), protected from moisture, was heated at 100° for 16–18 hr. Most of the volatile reactants were removed under red. press. and the red viscous residue poured into water (600 ml) and the mixture carefully heated with stirring to 80° to decompose the acetic anhydride. On cooling, an orange red solid separated, which was filtered, washed with several portions of 5 N HCl, then with ice-water and dried. It was then suspended in benzene (100 ml), warmed on the water-bath for a short time and filtered. The yellow residue was crystallized from a large volume of aqueous EtOH to give the *o*-nitrocinnamic acid (83 g; m.p. 145–151° after shrinking at 133°). Repeated crystallization from aqueous alcohol, ethyl acetate or acetic acid gave bright yellow prisms, m.p. 151–153° after preliminary shrinking and softening at 140°. (Found: C, 58·6; H, 4·8; C, 58·8; H, 4·7. $C_{18}H_{17}NO_7\frac{1}{2}H_2O$ requires: C, 58·7; H, 4·7%.)

(b) A mixture of homoveratric acid (6·72 g), 4-methoxy-2-nitrobenzaldehyde (4·97 g), acetic anhydride (30 ml) and anhydrous potassium acetate (0·62 g) was gently refluxed for 24 hr. The deep red solution was poured into ice-water. The solvent was removed *in vacuo* and the residue poured into K_2CO_3 aq and the mixture gently warmed to facilitate the dissolution of the gummy material. The clear red solution was extracted with benzene to remove all non-acidic material and the aqueous solution acidified with acetic acid. The yellow precipitate was filtered, washed well with water and dried. Repeated crystallization of the product from aqueous EtOH yielded bright yellow needles (3·2 g) of the *o*-nitrocinnamic acid, m.p. 149–151° with shrinking and softening at 140°. The m.p. could not be improved by chromatography, sublimation or crystallization.

2-Amino-4-methoxy-α-(3,4-dimethoxyphenyl) cinnamic acid

A hot solution of the above acid (20 g) in NH_4OH (14%; 800 ml) was added with vigorous stirring to the reduction mixture prepared from $FeSO_4$ (160 g), NH_4OH (600 ml; 0·88) and water (600 ml) at 85–90° during 10 min. After stirring at 90° for 1½ hr, the mixture was cooled to room temp and filtered from the iron sludge. The inorganic residue was thoroughly extracted with dil NH_4OH aq and the combined ammoniacal solution cooled and extracted thrice with $CHCl_3$ to remove non-acidic material. Acidification of the aqueous solution with acetic acid precipitated the *o*-aminocinnamic acid as a pale yellow solid, which was filtered, washed with water and dried. Crystallization from aqueous EtOH yielded pale yellow needles of the amino acid (14 g), m.p. 170–173°. On drying *in vacuo* at 80° for 6 hr the compound had m.p. 164–166° after slight shrinking at 165°. (Found: C, 66·0; H, 6·1. $C_{18}H_{19}NO_6$ requires: C, 65·66; H, 5·77%.)

1,2-Dihydro-3-(3,4-dimethoxyphenyl)-7-methoxy-2-oxoquinoline

The $CHCl_3$-extract from the last experiment was washed with water and dried (Na_2SO_4). Removal of solvent yielded a red gum which was chromatographed on Al_2O_3 and eluted with $CHCl_3$. Evaporation of the eluate gave a white solid (1·2 g) which was crystallized from $CHCl_3$–C_6H_6 to yield colourless needles of the carbostyril, m.p. 226–228°. Three crystallizations from EtOAC–$CHCl_3$ gave the analytically pure sample m.p. 232–234°. The IR spectrum (nujol) had an absorption band at 1653 cm^{-1} (NH–CO). (Found: C, 69·48; H, 5·48. $C_{18}H_{17}NO_4$ requires: C, 69·45; H, 5·46%.)

Methyl-3,6,7-trimethoxyphenanthrene-9-carboxylate

To a solution of the above amino acid (10 g) in dry acetone (400 ml) was added dropwise with stirring dil H_2SO_4 (20%; 53 ml). The amine sulphate that soon separated was diazotised at 0° with a solution of n-butyl nitrite (5 ml) in dry acetone (10 ml) during 15–30 min. After the addition was over, dry ice-cold acetone (200 ml) was added and the mixture kept stirred at 0° for ½–¾ hr. Freshly prepared Cu-powder (5 teaspoonful) was added when vigorous evolution of N_2 took place. The mixture was kept at 0–5° for 3 hr at 10–15° for 2 hr and then allowed to come to room temp and left overnight. The clear brown solution was filtered from the inorganic residue, the residue washed on the filter with dry hot acetone and the combined filtrates evaported on the water bath. Addition of water precipitated the phenanthrene acids as brown needles which were filtered and dried. The crude mixture of acids was dissolved in dil NH_4OH aq and extracted with $CHCl_3$ to remove non-acidic material. The ammoniacal solution was acidified with conc. HCl and the gummy precipitate on keeping at 0° overnight solidified. The solid was filtered, washed free of HCl with ice-water and dried *in vacuo* at 60° for 7 hr. The crude acid (6·6 g) was directly esterified by refluxing with dry MeOH (210 ml) in the presence of conc. H_2SO_4 (5·3 ml) for 6 hr. The dark brown solution was concentrated (ca. 70 ml of MeOH was removed) and allowed to cool overnight at room temp. The crystalline solid which had separated was filtered and dried (MeOH filtrate—see next experiment). It was then taken up in C_6H_6 and washed successively with dil. NH_4OH aq, H_2O and dried (Na_2SO_4). Removal of solvent and crystallization of the residue from MeOH gave pale brown needles of methyl-3,6,7-trimethoxyphenanthrene-9-carboxylate (4·3 g) m.p. 144–147°. Chromatography on Al_2O_3, elution with C_6H_6, evaporation and crystallization of the residue twice from MeOH gave colourless needles of the methyl ester m.p. 149–151°. (Govindachari *et al.*,[2] reported m.p. 155°). The IR spectrum of the compound (CH_2Cl_2) showed a sharp peak at 1702 cm^{-1} (aromatic ester). (Found: C, 70·08; H, 5·50. $C_{19}H_{18}O_5$ requires: C, 69·93; H, 5·52%.)

Methyl-3,5,6-trimethoxyphenanthrene-9-carboxylate

The original methanol filtrate was concentrated *in vacuo* to a small bulk, treated with dil. NH_4OH aq and extracted with $CHCl_3$. Removal of solvent and crystallization of the residue from MeOH gave the ester (1·2 g), m.p. 94–98°. Chromatography on Al_2O_3, elution with 1:1 C_6H_6–pet. ether (40–60°), evaporation and crystallization of the residue from pet. ether (40–60°)–C_6H_6 yielded colourless needles of methyl 3,5,6-trimethoxyphenanthrene-9-carboxylate m.p. 104–106°. Two crystallizations from the same solvent pair gave the analytically pure sample, m.p. 106·5–107° (λ_{max}^{EtOH} 250, 276 (sh), 332, 354 (sh), 372 mμ Log ε 4·78, 4·36, 4·17, 3·95, 3·64). The IR spectrum of the compound (CH_2Cl_2) showed a sharp band at 1700 cm^{-1} (aromatic ester). (Found: C, 69·72; H, 5·59. $C_{19}H_{18}O_5$ requires: C, 69·93; H, 5·52%.)

3,6,7-Trimethoxyphenanthrene-9-carboxylic acid

A solution of methyl 3,6,7-trimethoxyphenanthrene-9-carboxylate (0·3 g) in alcoholic KOH (10%, 6 ml) was refluxed on a water bath. At the end of 2 hr, the K-salt of the acid began to crystallize. The mixture was refluxed for a total period of 3 hr and then enough water was added to get a clear solution. The solution was concentrated to a small bulk and water (10 ml) added. The solution was extracted with C_6H_6 and the aqueous layer acidified with dil. HCl. The precipitate was filtered, washed with water and dried. Two crystallizations from MeOH gave feathery white needles of the acid (0·21 g) m.p. 212–215° after slight shrinking at 208°. The mixed m.p. with the authentic sample (Govindachari *et al.*, loc. cit) was 211–214°.

3,5,6-Trimethoxyphenanthrene-9-carboxylic acid

Hydrolysis of methyl 3,5,6-trimethoxyphenanthrene-9-carboxylate (0·2 g) as above, gave after two crystallizations from C_6H_6, the corresponding acid (0·12 g) as colourless cubes, m.p. 204–205°.

(λ_{max}^{EtOH} 250, 257, 272, 316, 372 mμ log ε 4·61, 4·51, 4·24, 4·08, 3·5). The IR spectrum of the compound (nujol) had a strong band at 1670 cm^{-1} (COOH). (Found: C, 69·52; H, 5·08. $C_{18}H_{16}O_6$ requires: C, 69·23; H, 5·12%.)

9-Hydroxymethyl-3,6,7-trimethoxyphenanthrene

Govindachari *et al.*, loc. cit.

9-Chloromethyl-3,6,7-trimethoxyphenanthrene

Govindachari *et al.*, loc. cit. The m.p. 147° remained undepressed on admixture with the authentic sample, the m.p. of which had been erroneously reported as 163–164°.

Methyl-3,6,7-trimethoxy-N-(9-phenanthrylmethyl)-pyrrolidine-2-carboxylate

Methyl-L-prolinate hydrochloride was first prepared as follows:

A solution of L-proline (2·5 g) in dry MeOH (37·5 ml), protected from moisture, was saturated at 0° with dry HCl. The mixture was allowed to stand at room temp for 20 hr. Most of the MeOH was then removed *in vacuo* at room temp and the oily residue again dissolved in dry MeOH (37·5 ml) and resaturated with dry HCl at 0°. After remaining at room temp for 20 hr, the solution was taken to dryness *in vacuo* at room temp. The residue was washed thrice with dry ether and dried *in vacuo* over NaOH for 3 days, when the oil crystallized to give long needles of methyl-L-prolinate hydrochloride.

The above ester hydrochloride was suspended in dry dioxane (50 ml), cooled in ice water and treated with freshly ignited K_2CO_3 (3·5 g). The mixture, protected from moisture, was ground well to liberate the free base. A pinch of NaI followed by a hot solution of the above chloride (1·25 g) in dry dioxane (30 ml) was added and the yellow solution stirred vigorously at room temp for 15 min. It was then heated with stirring at 100° on the water bath for 7 hr. The solution was filtered hot and the filtrate evaporated to dryness under red. press. The red residue was thoroughly extracted with several portions of boiling ether. The ether solution was repeatedly extracted with dil. HCl (100 ml H_2O + 20 ml conc. HCl). The aqueous acidic extract was cooled to 10° and basified with dil. NH_4OH aq. The base was re-extracted with ether, washed with water and dried (Na_2SO_4). Removal of solvent gave a white residue (1·2 g) which was purified by chromatography on Al_2O_3 and elution with C_6H_6. Evaporation of the eluate and crystallization of the residue from MeOH gave colourless needles of the N-alkylated ester (0·93 g) m.p. 145–147°. (λ_{max}^{EtOH} 240 (sh), 268, 284, 298 (sh), 308 (sh) mμ Log ε 4·57, 4·81, 4·55, 4·28, 3·93). The IR spectrum of the compound (nujol) showed a sharp peak at 1735 cm^{-1} ($COOCH_3$). (Found: C, 70·33; H, 6·63. $C_{24}H_{27}NO_5$ requires: C, 70·42; H, 6·6%.)

3,6,7-Trimethoxy-N-(9-phenanthrylmethyl) proline hydrochloride

A solution of the above amino ester (1·7 g) in conc. HCl (20 ml) was gently refluxed in an oil-bath for 1 hr. Excess of HCl was removed under red. press. and the residue treated with dry acetone. The white solid that separated was filtered and washed well with acetone. Crystallization of the residue (1·4 g) from EtOH–dry acetone gave white plates of the amino acid hydrochloride (1·02 g) m.p. 208–210° (dec). (λ_{max}^{EtOH} 240, 260, 284, 316 (sh), 342, 358 mμ Log ε 4·46, 4·73, 4·50, 3·93, 3·14, 2·78. (Found: C, 63·72; H, 6·02. $C_{23}H_{26}NO_5Cl$ requires: C, 63·98; H, 6·0%.)

9,11,12,13,13a,14-Hexahydro-3,6,7-trimethoxy-14-oxodibenzo (f, h) pyrrolo (1,2-b) isoquinoline

The above amino acid hydrochloride (0·98 g) [previously dried at 100° over P_2O_5 *in vacuo* for 7 hr] was added in one lot to polyphosphoric acid (6·13 g) and the mixture protected from moisture, was vigorously stirred at 100° in an atm of pure dry N_2 for 3½ hr. The reddish-brown reaction mixture was poured into ice-water and the greenish yellow solution filtered from a small amount of an insoluble material. The filtrate was cooled to 0° and basified with KOH aq (50%). The liberated base was extracted with $CHCl_3$ (7 × 30 ml), washed with sat. NaCl aq and dried (Na_2SO_4). The solvent was removed *in vacuo* to give a pale yellow solid (0·17 g) m.p. 163–166° (dec). The IR spectrum of the compound ($CHCl_3$) showed a strong band at 1670 cm^{-1} indicating the presence of an aromatic carbonyl group. The ketone was reduced as such without further purification.

9,11,12,13,13a,14-Hexahydro-14-hydroxy-3,6,7-trimethoxy benzo (f, h) pyrrolo (1,2-b) isoquinoline

A suspension of the above crude ketone (0·17 g) in a mixture of dry dioxane (35 ml), dry tetrahydrofuran (20 ml) and MeOH (20 ml) was treated with an excess of $NaBH_4$ (0·25 g) and the mixture gently refluxed in an atm. of N_2 on a water bath for ¾ hr. The colourless solution was allowed to cool and the solvent removed *in vacuo* at room temp. The residue was treated with water, filtered and dried. The IR spectrum of the crude reduction product (0·13 g; m.p. 236–242° (dec)) in $CHCl_3$ showed no carbonyl absorption. Thin layer chromatographic examination of the product revealed it to be a mixture of two main compounds from which one pure material was isolated by column chromatography on Al_2O_3 (30 g) and elution with 0·5% methanolic benzene. (The material was dissolved in dry $CHCl_3$ (8 ml) and was adsorbed on the alumina column which was washed free of $CHCl_3$ by running in dry C_6H_6 (40 ml) and draining.) The material was then eluted with 0·5% MeOH–C_6H_6 and fractions were cut at each 5 ml of the eluate.

Eluate	Residue	No. of Spots*
(5 ml fractions)		
1–27	nil	—
28		
29–35	mainly ± tylophorinine and trace of impurity	2
36–63	35 mg	2

* Chromatogram was developed with I_2 vapour.

Fractions 29–35 were combined and evaporated to give a pale yellow material† (0·046 g) which was rechromatographed on Al_2O_3 (8·5 g) and eluted with 1% MeOH–C_6H_6 and fractions cut at each 3 ml of the eluate. Fractions 8–11 in this chromatography were again combined and evaporated. TLC examination on Al_2O_3 plate showed it to be virtually homogenous. Crystallization from $CHCl_3$–MeOH gave white microcrystalline material m.p. 246° (dec) with slight sintering at 243°. (Found: C, 72·68; H, 6·6. $C_{23}H_{25}O_4N$ requires: C, 73·8; H, 6·6%.) It may be noted that correct analytical values could not be obtained[3] for the natural base either.

The free base (0·022 g) was dissolved in dry $CHCl_3$ (4 ml) and the solution cooled to 0°. Dry HCl was passed through until saturation and the hydrochloride precipitated completely by the addition of dry ether. The solid was centrifuged, washed several times with dry ether and crystallized twice from MeOH to give (±)-tylophorinine HCl as pale yellow cubes m.p. 249–252° (dec). (Found: C, 66·88; H, 6·28. $C_{23}H_{25}O_4N.HCl$ requires: C, 66·44; H, 6·25%.)

Acknowledgement—We thank the Council of Scientific & Industrial Research for award of a Senior Research Fellowship to S. P. and Junior Research Fellowship to T. S. S.

† TLC examination revealed a considerable enrichment of one diastereoisomeric racemate.

[3] T. R. Govindachari, B. R. Pai and K. Nagarajan, *J. Chem. Soc.* 2801 (1954).